



Hydroxypyridinecarboxylic acid derivatives influencing metal ion levels in the brain: Equilibrium complexation studies with Cu(II) and Zn(II)



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ABSTRACT

The metal ion chelators 4-hydroxy-5-methyl-3-pyridinecarboxylic acid (DQ5) and 1,5-dimethyl-4-hydroxy-3-pyridinecarboxylic acid (DQ715) and Cu(II) and Zn(II) were investigated with the aim to restore the homeostasis of the brain Cu(II) and Zn(II) in neurodegenerative diseases. The proton dissociation constants of the ligands, the stability constants, and the coordination modes of the metal complexes formed were determined by pH-potentiometric, and spectral (UV–Vis and EPR or ¹H NMR) methods. The results show that in slightly acidic and neutral pH range mono and bis complexes are formed through bidentate coordination of the ligands. The biological MTT-test reveals that the DQ715 ligand is able to lower the cytotoxic effect of Cu(II) in human embryonic kidney HEK-293 cells. Our studies revealed, however, that none of the chelators were efficient enough to withdraw these metal ions from the amyloid aggregates.

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1. Introduction

Balanced homeostasis of metal ions is critical for the normal function of the brain and is maintained within strict limits [1]. Disharmony in metal ion homeostasis, especially that of iron and copper will cause oxidative stress by increasing the formation of reactive oxygen species (ROS) as superoxide ion, hydrogen peroxide, and hydroxyl radical, and thus damage many biomolecules in the cells resulting in various neurodegenerative disorders [2].

It has been demonstrated that the increased brain Cu(II), Fe(III) and also Zn(II) concentration in dyshomeostasis of these metal ions influences the oligomerisation of β -amyloids in the Alzheimer's brain [3]. The interactions of these metal ions with amyloid precursor (APP) or amyloid β -peptide (A β) can produce neurotoxic H₂O₂. Then the reduced metal ions reacting with hydrogenperoxide will generate the extremely reactive hydroxyl radicals from hydrogen peroxide in $M(\text{red}) + \text{H}_2\text{O}_2 \rightarrow M(\text{ox}) + \text{OH}^\cdot + \text{OH}^-$ reaction leading to oxidative stress in brain [4–6]. Some selective ligands for Cu(II) have been proposed as chelating agents for the therapy of

AD. The first was clioquinol (CQ), which could chemically solubilize A β deposits in AD [7] likely through the interaction with copper. Fallor and coworkers suggested that a chelator with a conditional dissociation constant (K_D) of 10 pM (up to 100 nM range) for Cu(II) should be sufficient to retrieve copper completely from amyloid deposits [8]. Other results show that an α B-crystallin chaperon peptide prevents Cu(II)-induced aggregation of A β _{1–40} due to selective Cu(II) binding ability in addition to preventing the amyloid fibril formation of A β peptides [9]. The dissociation constant (K_D) for Cu(II) interaction with the chaperon peptide is in the μM range [9,10].

The role of Zn this inert trace element is much less clear in the neurodegenerative processes. Reported Zn(II) affinity for A β is significantly weaker than, that for Cu(II), values of dissociation constants ranging between 1 and 20 μM [9]. However, the amyloid aggregates contain relatively high concentration (mM) of zinc [11]. Several attempts were made to obtain efficient chelators with moderate affinity towards the metal ions such as Cu(II), Zn(II) or Fe(III) that participate in amyloid aggregation, in order to prevent the formation of plaques [12–14].

In previous papers [15–20] we reported the evaluation of several hydroxypyridinecarboxylic acid derivatives (HPCs) as possible chelating agents for Fe(III) and Al(III). To this aim, the Fe(III) and Al(III) complexes formed with selected HPCs were studied. Now

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we extended these studies also to Cu(II) and Zn(II). On one hand, it is well known that any Fe(III) and Al(III) chelator can complex essential metal ions too in a chelation therapy regiment, thus causing toxic side effects due to metal ion deficiency. For example, zinc deficiency problems are sometimes experienced in the deferiprone therapy [21,22]. The evaluation of the complexation strength of HPCs towards Cu(II) and Zn(II) can allow to predict the extent of essential metal ion removal during the Fe(III) and Al(III) chelation therapy. On the other hand, the very low cytotoxicity of HPCs, and according to the Lipinski's rule their low molecular mass, the reasonable lipophilicity (which can allow the oral activity and the easy blood brain barrier crossing) can represent important advantages also for the employment of HPCs in the AD therapy. Therefore, the evaluation of the complexation strength of the HPCs towards Cu(II) and Zn(II) can allow to predict if these ligands can remove copper and zinc from A β , *i.e.* if they represent good candidates also in the recovery of the disturbed brain metal ion homeostasis in AD.

In the present paper we studied the Cu(II) and Zn(II) binding affinity of two HPCs derivatives: 4-hydroxy-5-methyl-3-pyridinecarboxylic acid (DQ5) and 1,5-dimethyl-4-hydroxy-3-pyridinecarboxylic acid (DQ715) (Scheme 1). Their coordination properties in aqueous solution were determined by means of pH-potentiometric titrations, UV-Vis and ¹H NMR or EPR measurements. The effects of DQ715 with Cu(II) chloride were evaluated on cell viability of a combined way in human embryonic kidney HEK-293 cells.

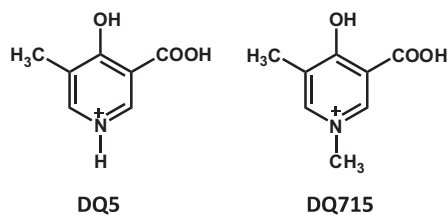
2. Experimental

2.1. Chemicals

DQ5 and DQ715 were synthesized as described in Ref [15]. Double-distilled Milli-Q water was used for sample preparations. The purity of the ligands was checked and the exact concentrations of the stock solutions prepared were determined by potentiometric titrations using the program SUPERQUAD for data evaluation [23]. The pH-metric titrations were performed with 0.1 mol/dm³ KOH prepared from KOH (Merck). The base was standardised against HCl solutions prepared from 36% HCl (Merck). A ZnCl₂ stock solution was made by dissolution of anhydrous ZnCl₂ in a known amount of HCl, and its concentration was determined by complexometry via ethylenediaminetetraacetate complexes, and gravimetrically via the oxinate. The CuCl₂ ion stock solutions were prepared from CuCl₂·2H₂O (Reanal) dissolved in doubly distilled water, and the concentration of the metal ion was determined gravimetrically via precipitation of the oxinate.

2.2. pH-potentiometric studies

The pH-metric measurements for determining stability constants of the proton and metal complexes of the ligands were carried out in aqueous solution at an ionic strength of 0.2 mol/dm³ KCl (Sigma Aldrich) at 25.0 ± 0.1 °C. The titrations were performed



Scheme 1. 4-Hydroxy-5-methyl-3-pyridinecarboxylic acid (DQ5) and 1,5-dimethyl-4-hydroxy-3-pyridinecarboxylic acid (DQ715) shown in their fully protonated forms (H₃L⁺ and H₂L⁺, respectively).

with a carbonate-free KOH solution of known concentration (ca. 0.1 mol/dm³). In order to keep the ionic strength constant KCl has been added to the KOH solution to set the K⁺ concentration 0.2 mol/dm³. The HCl concentration was determined by potentiometric titrations using the Gran's method [24]. An Orion 710A pH-meter equipped with a Metrohm combined electrode (type 6.0234.1000) and a Metrohm 665 Dosimat burette was used for the pH-metric measurements. The electrode system was calibrated according to Irving et al. [25] (strong acid versus strong base; HCl versus KOH titration) and therefore the pH-meter readings could be converted into hydrogen ion concentration. The water ionization constant, pK_w calculated from strong acid-strong base titrations was 13.76 ± 0.01 under the conditions employed. The titrations were performed in the pH range 2–11 or until precipitation occurred in the samples. The initial volume of the samples was 10 cm³ in case of DQ715 and 20 cm³ in case of DQ5 related titrations. The ligand concentration was in the range of 0.5 × 10⁻³–2 × 10⁻³ mol/dm³, and the metal ion to ligand ratios were 1:1, 1:2 and 1:4. The accepted fitting between the experimental and the calculated titration curves was always better than 0.01 cm³ and the uncertainties (3SD values) in the stability constants are given in parentheses in Table 1. The samples were in all cases deoxygenated by bubbling purified argon for ca. 10 min before the measurements, and argon was also passed through the solutions during the titrations.

The protonation constants of the ligands were determined with the computer program SUPERQUAD [23]. PSEQUAD [26] was used to establish the stoichiometry of the complexes and to calculate their stability constants (log β (M_pL_qH_r)). β (M_pL_qH_r) is defined for the general equilibrium reaction pM + qL + rH ⇌ M_pL_qH_r as β (M_pL_qH_r) = [M_pL_qH_r]/[M]^p[L]^q[H]^r, where M denotes the metal ion, L is the completely deprotonated ligand molecule, and p, q and r are the number of metal, ligand, and proton atoms, respectively. According to the calibration protocol employed, the protonation and stability constants are concentration constants which refer to the given ionic strength. The calculations were always made from the experimental titration data measured in the absence of any precipitate in the solution.

2.3. Spectrophotometric measurements

UV-Vis spectrophotometric measurements were performed in aqueous solution at 25.0 ± 0.1 °C on solutions containing the ligand (either DQ715 or DQ5) at a 8.0 × 10⁻⁵ mol/dm³ concentration, and the metal (either Cu(II) or Zn(II)) at the following metal to ligands ratios: 0:1, 1:4, 1:2, 1:1. The pH range was from 2 to 11, and the ionic strength was 0.20 mol/dm³ (KCl). The spectra were recorded under argon atmosphere. A Hewlett Packard 8452A diode array spectrophotometer was used to record the UV-Vis spectra in the interval 290–820 nm. The pathlength was 1 cm using quartz cuvettes. Protonation and stability constants and the individual spectra of the species were calculated by the computer program PSEQUAD [26].

2.4. ¹H NMR measurements

¹H NMR studies were carried out on a Bruker Ultrashield 500 Plus instrument equipped with a 5 mm capillary NMR tube. In the NMR measurements the magnetic field was stabilised by locking with the ²D signal of the solvent. The sample temperature was set to 25 ± 1 °C during all data acquisitions. Chemical shifts are reported in ppm (δ_H) from 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS) as internal reference. The ¹H NMR measurements were performed with WATERGATE solvent suppression scheme. All samples were measured with the same experimental parameters, the same spectrometer and the same probe. The relaxation delay, the delay for binomial water suppression, and the number of scans,

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